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Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States)

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Abstract

Objective: Risks of some cancers in adults have been associated with several pregnancy factors, including greater maternal age and birth weight. For hormone-related cancers, these effects are hypothesized to be mediated through higher *in utero* estrogen concentrations. In addition, racial differences in pregnancy hormone levels have been suggested as being responsible for differences in testicular and prostate cancer risk by race. However, data on hormonal levels related to these characteristics of pregnancy are sparse, particularly those from studies of the fetal circulation.

Methods: Estrogen and androgen concentrations were measured in maternal and umbilical cord sera from 86 normal, singleton pregnancies.

Results: Birth size measures (weight, length and head circumference) were positively correlated with maternal estriol ($r = 0.25$–0.36) and with cord DHEAS concentrations ($r = 0.24$–0.41), but not with estrogens in cord sera. Maternal age was inversely correlated with maternal DHEAS, androstenedione and testosterone concentrations ($r = -0.30$, -0.25 and -0.30, respectively), but uncorrelated with estrogens in either the maternal or cord circulation. Black mothers had higher androstenedione and testosterone concentrations than white mothers, however, there were no racial differences in any of the androgens in cord sera. Cord testosterone concentrations were higher in mothers of male fetuses, while both maternal and cord concentrations of estriol were lower in these pregnancies.

Conclusions: These data demonstrate associations between hormone concentrations and pregnancy factors associated with offspring's cancer risk, however, the hormones involved and their patterns of association differ by whether the maternal or fetal circulation was sampled. Hormone concentrations in the fetal circulation in this study are not consistent with the hypothesis that greater estrogen concentrations in high birth weight babies mediate the positive association with breast cancer risk observed in epidemiologic studies, or with the hypothesis that higher testosterone exposure in the *in utero* environment of black males explains their higher subsequent prostate cancer risk.

Introduction

Elevated concentrations of pregnancy estrogens or androgens have been speculated to increase risk of breast cancer in daughters [1], and testicular and prostate cancer in sons [2, 3]. Epidemiologic studies provide some evidence of altered cancer risks in offspring with several

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maternal, perinatal and pregnancy characteristics thought to be markers for pregnancy hormone levels including, preeclampsia [4], maternal age [4] and birth weight [5]. Differences in pregnancy hormone concentrations related to these risk factors have been noted, though data are sparse. Furthermore, studies have focused primarily on estrogens [6–10]. Androgen exposure *in utero* also might be relevant, possibly conferring long-term protection against breast cancer by antagonizing effects of estrogens on fetal breast ductal development [11].

To date, however, studies have relied on hormone concentrations measured in the maternal circulation to characterize fetal exposure while little is known about the relationships of pregnancy factors to hormone concentrations in cord blood. We examined associations of several maternal, perinatal and pregnancy factors, including maternal age and race, and birth weight, with maternal and umbilical cord estrogen and androgen concentrations in normal, uncomplicated pregnancies.

Methods and materials

Study population

Subjects were a sample from an ongoing study of preeclamptic pregnancies being conducted at the Magee Womens Hospital, University of Pittsburgh. All women attending the Magee Womens Hospital's obstetric practice who delivered between February 1994 and May 1998, and were 14 years of age or older were invited to participate in the study as controls; 52% agreed to participate. Women pregnant with more than one fetus or who had pregestational diabetes or hypertension were excluded. A control was chosen for each preeclampsia case that matched as closely as possible on parity, length of pregnancy at delivery, type of delivery and maternal age (± 5 years). In the present analysis, the control group of 86 women was used to assess the relationship between hormone levels and pregnancy-related variables in women with normal pregnancies. Informed consent for the questionnaire, interview and blood collection was obtained from all study participants.

The women were in their mid-twenties, on average ($x = 26.2$ years). Mean length of gestation was 37.8 weeks although the median was closer to 39 weeks. This was because in the initial study, subjects were matched to preeclampsia cases by length of pregnancy, and cases tended to deliver before 40 weeks. Mean birth weight was 3165 g (median = 3252 g). Only two of the women identified themselves as not being white or black, and these women were excluded from the analyses involving a comparison of white and black women.

Hormone assays

Maternal serum was collected at admission for labor and delivery and mixed venous and arterial cord serum was collected at delivery. The samples were allowed to clot at room temperature, were centrifuged and stored at −80 °C. Blood samples were analyzed at Quest Diagnostics, San Juan Capistrano, CA. Unconjugated concentrations of estrone (E_1), estradiol (E_2) and androstenedione were measured by an in-house method of radioimmunoassay (RIA) following extraction with organic solvent and purification by celite chromatography [12, 13]. Testosterone and estriol (E_3) were measured by extraction and RIA and DHEA sulfate (DHEAS) by dilution and RIA. Blinded aliquots of pooled sera from normal, pregnant women constituted 10% of each batch of study samples. The coefficients of variation based on blinded quality control samples for the maternal hormones were 18.6% for DHEA, 8.5% for DHEAS, 10.2% for androstenedione, 9.6% for testosterone, 13.7% for estradiol, 10.3% for estrone and 6.8% for estriol and 8.1, 6.6, 8.5, 15.2, 10.9, 16.7, and 9.2%, respectively, for hormones in cord blood. Excluding batches with values over 2 SD from the batch mean did not affect the results presented.

Information on maternal age, race or ethnicity, menstrual, reproductive and medical history, pre-pregnancy weight, smoking anytime during pregnancy and length of gestation was obtained by interview and supplemented by subjects' medical records. These supplemental data included blood pressure measurements, blood and urine work-ups, delivery method, medication use during labor, and the baby's sex and birth anthropometrics.

Statistical analysis

Spearman rank correlations were calculated between maternal and cord blood hormone values and pregnancy factors because the hormones were not normally distributed. Mean maternal and cord hormone concentrations were compared by categories of maternal, pregnancy and perinatal factors using analysis of covariance with logarithm-transformation of all hormones. Categories of maternal height and pre-pregnancy weight, birth length and head circumference were based on tertiles. Geometric means are presented.

To evaluate the independence of the observed associations, factors associated with the hormones in the correlations and analyses of variance were entered as independent variables in linear regression models with the logarithm-transformed hormones as dependent variables. Models for hormones associated with factors

not of etiological significance (timing of blood draw, delivery method, duration of labor and use of oxytocin) were repeated excluding these variables if their adjustment did not influence the associations of the hormones with the variables of interest. Birth weight was not included simultaneously with birth length or head circumference in the regression models because of high correlations ($r = 0.70$ with birth length; $r = 0.69$ with head circumference). Gestational age was included in models that included birth weight whether or not it was statistically significant in univariate analyses with the hormones because of the possibility of residual confounding. Maternal age, height and pre-pregnancy weight, birth weight, and weeks of gestation were treated as continuous. The anti-logs of the β coefficients are presented. Statistical significance was defined at the $p < 0.05$ level.

Results

Table 1 presents correlations between pregnancy factors and maternal and cord hormone concentrations, and Tables 2 and 3 present mean hormone concentrations by pregnancy factors in maternal and cord serum, respectively. In general, more statistically significant and stronger associations were observed between pregnancy factors and maternal hormone concentrations than with cord hormone concentrations. Furthermore, maternal factors, such as age, race and body size were more likely to be associated with maternal hormones than with cord hormones, while characteristics of the fetus were more likely to be associated with fetal hormonal measures than with those of the mother.

Gestational age ($r = 0.35$) and birth size including, birth weight ($r = 0.36$), length ($r = 0.30$) and head circumference ($r = 0.25$) were positively correlated with maternal estriol (Table 1). Maternal age inversely correlated with maternal androstenedione ($r = -0.25$), DHEAS ($r = -0.30$) and testosterone ($r = -0.30$) concentrations, whereas maternal height ($r = 0.21$) and pre-pregnancy weight ($r = 0.22$) positively correlated with maternal testosterone. The associations of pregnancy factors with hormone concentrations in the cord were not always of the same magnitude or in the same direction as those observed with the maternal hormones. Gestational age and birth size correlated positively and statistically significantly only with cord DHEAS concentrations ($r = 0.24$–0.41) (Table 1).

Mean maternal estrogens appeared to be lowest in the mothers with the lightest and shortest babies, with no consistent rise with increasing birth weight or length,

Table 1. Spearman correlations between maternal and cord serum hormone levels and maternal, gestational and neonatal characteristics

	Andro	Testos	DHEA	DHEAS	Estradiol	Estriol	Estrone
Gestational age							
Cord	0.02	0.12	0.10	0.32*	−0.07	0.01	0.01
Maternal	−0.04	−0.06	−0.12	−0.14	0.04	0.35*	−0.03
Birth weight							
Cord	−0.05	0.09	0.13	0.34*	−0.08	−0.08	0.01
Maternal	−0.00	−0.07	−0.00	−0.02	0.13	0.36*	0.00
Birth length							
Cord	0.10	0.17	0.10	0.41*	−0.03	0.06	0.06
Maternal	−0.01	−0.06	0.12	−0.02	0.02	0.30*	−0.04
Head circumference							
Cord	−0.06	0.09	0.09	0.24*	−0.05	−0.04	−0.04
Maternal	−0.05	−0.08	−0.01	−0.03	0.09	0.25*	−0.09
Maternal age							
Cord	−0.01	0.10	0.18	0.18	0.02	0.11	−0.00
Maternal	−0.25*	−0.30*	−0.09	−0.30*	−0.00	0.16	0.02
Pre-pregnancy weight							
Cord	−0.06	0.07	−0.18	−0.15	−0.08	−0.10	−0.07
Maternal	0.16	0.22*	−0.04	−0.08	−0.09	−0.11	−0.19
Mother's height							
Cord	0.01	−0.03	−0.05	0.02	−0.05	−0.04	0.04
Maternal	0.17	0.21*	0.04	0.02	0.05	−0.04	−0.05

Andro: androstenedione; Testos: testosterone.
* $p \leq 0.05$.

Table 2. Mean maternal serum hormone concentrations by maternal, gestational and neonatal characteristics in 86 uncomplicated pregnancies

	N	Andro (ng/dl)	Testos (ng/dl)	DHEA (ng/dl)	DHEAS (ug/dl)	Estradiol (pg/ml)	Estriol (ng/ml)	Estrone (pg/ml)
Gestational age (weeks)								
< 38	24	352	152	463	106	18,644	12.2[2,3]	5436
38–39	47	343	149	492	87.9	23,119	18.5[1]	7746
40 +	15	352	141	434	85.7	21,668	18.6[1]	6038
Birth weight (g)								
< 2500	7	246	126	276	54.9	11,558[2,3]	9.1[2,3]	3144[2,3]
2500–3499	56	361	158	499	98.4	22,567[1]	16.9[1]	7569[1]
3500–4499	23	349	134	490	91.9	23,191[1]	18.7[1]	6333[1]
Birth length (cm)								
< 49	26	324	145	365[2]	84.5	18,135[2]	13.2[2,3]	5488[2]
49–51	27	385	167	568[1]	111.6	24,708[1]	17.7[1]	8306[1]
51 +	31	342	137	522	87.9	21,880	18.6[1]	6638
Head circumference (cm)								
< 33.1	27	356	155	490	101	20,776	14.5[3]	7173
33.1–34.5	22	422	179	507	94.5	19,241	15.0	6631
34.5 +	34	312	131	464	89.9	23,603	19.4[1]	6386
Sex								
Female	37	314	131	469	86.0	23,049	18.5*	7640
Male	49	374	162	477	97.1	20,445	15.1*	6098
Maternal age (years)								
< 20	13	373[5]	178[5]	414	89.7	13,189[2–4]	11.4[2–5]	4484[2]
20–24	24	429[5]	188[5]	581	133.8[4,5]	27,255[1,5]	16.8[1]	8496[1]
25–29	25	352[5]	143[5]	483	99.1[5]	23,058[1]	18.3[1]	7254
30–34	11	333	151[5]	494	70.4[2]	24,394[1]	18.6[1]	7113
35 +	13	220[1–3]	84.8[1–4]	345	51.9[2,3]	17,918[2]	17.2[1]	5368
Race								
White	50	295*	117*	424	88.8	22,584	17.5	6380
Black	34	447	215	554	99.4	20,035	15.0	7166
Maternal height (cm)								
< 160	21	292	119[3]	475	95.6	20,499	16.6	7415
160–165	32	355	148	509	92.5	23,740	18.5	6446
165 +	33	379	171[1]	440	89.7	20,197	14.7	6569
Pre-preg. weight (kg)								
< 59.1	28	300	122[3]	441	85.0	18,953	15.1	6660
59.1–72.5	28	349	146	521	112	25,175	17.9	7885
72.5 +	29	390	178[1]	455	79.5	20,288	16.3	5715
Gravidity								
1	35	373	154	502	108	19,977	14.3*	6176
> 1	51	330	144	455	82.9	22,660	18.2*	7119
Parity								
0	63	357	153	485	102*	21,670	16.1	6659
1 +	23	321	135	442	69.1*	21,141	17.7	6885
Smoking								
Yes	23	338	151	463	87.6	21,235	16.6	7142
No	62	382	145	508	108	22,690	16.5	5867

Andro: androstenedione; Testos: testosterone.

* $p \leq 0.05$; [1] indicates significant difference at $p < 0.05$ with category 1; [2] indicates significant difference with category 2; [3] indicates significant difference with category 3.

Table 3. Mean cord serum hormone concentrations by maternal, gestational and neonatal characteristics in 86 uncomplicated pregnancies

	N	Andro (ng/dl)	Testos (ng/dl)	DHEA (ng/dl)	DHEAS (ug/dl)	Estradiol (pg/ml)	Estriol (ng/ml)	Estrone (pg/ml)
Gestational age (weeks)								
< 38	24	320	16.8	445	139[2,3]	8698	186	28,698
38–39	47	337	22.6	506	185[1]	9540	233	31,482
40 +	15	334	20.6	568	246[1]	7917	181	30,939
Birth weight (g)								
< 2500	7	246[2]	11.2[2,3]	268[2,3]	73.8[2,3]	4809[2]	165	12,791[2,3]
2500–3499	56	347[1]	20.7[1]	526[1]	189[1]	10,164[1]	222	34,101[1]
3500 +	23	326	23.8[1]	529[1]	208[1]	8034	193	30,577[1]
Birth length (cm)								
< 49	26	282[2]	15.1[2,3]	413[2,3]	129[2,3]	7421	170[2]	22,848[2]
49–51	27	362[1]	22.1[1]	548[1]	176[1]	10,596	268[1]	37,283[1]
51 +	31	340	22.9[1]	543[1]	235[1]	8543	201	31,684
Head circumference (cm)								
< 33.1	27	316	16.9	466	156	7571[2]	196	26,615[2]
33.1–34.5	22	376	21.8	496	174	12,586[1,3]	240	41,408[1,3]
34.5 +	34	304	20.8	523	201	7505[2]	197	26,508[2]
Sex								
Female	37	321	16.4*	520	176	8201	254*	31,809
Male	49	340	24.1*	484	183	9617	180*	29,684
Maternal age (years)								
< 20	13	322	16.6	384[2,5]	128[4,5]	7791	177	27,199
20–24	24	357	21.9	576[1]	185	10,762	225	35,727
25–29	25	321	19.2	450	176	7622	189	29,391
30–34	11	354	26.4	546	230[1]	9342	227	34,458
35 +	13	300	20.1	561[1]	205[1]	9824	239	25,168
Race								
White	50	332	20.6	502	171	9605	214	31,904
Black	34	338	19.9	492	188	8314	206	29,930
Maternal height (cm)								
< 160	21	338	24.2	537	190	9734	220	29,226
160–165	32	351	18.6	514	176	9757	233	33,973
165 +	33	310	20.0	462	178	7871	181	28,421
Pre-preg. weight (kg)								
< 59.1	28	339	19.5	507	191	8917	207	29,348
59.1–72.5	28	341	20.7	518	183	10,091	229	38,028
72.5 +	29	310	20.3	464	165	7666	186	24,416
Gravidity								
1	35	356	24.2	487	169	10,628	190	31,765
> 1	51	316	18.2	507	188	7999	222	29,792
Parity								
0	63	347	21.1	497	178	9902	201	33,662
1 +	23	293	18.6	506	185	6872	232	23,508
Smoking								
Yes	23	328	20.3	496	181	8809	204	28,866
No	62	338	20.5	502	177	9254	220	35,739

Andro: androstenedione; Testos: testosterone.

* $p \leq 0.05$; [1] indicates significant difference at $p < 0.05$ with category 1; [2] indicates significant difference with category 2; [3] indicates significant difference with category 3.

although there was limited data in the lightest birth weight category (Table 2). When examined by 5-year age groups, maternal androgens were generally statistically significantly lower only in the oldest maternal age group (35+ years) compared with the other categories. In contrast, maternal estradiol and estriol were lowest only in the youngest age group compared with the older age groups. As in the correlations, mother's height and pre-pregnancy weight were positively associated with maternal testosterone. Black mothers had higher maternal androstenedione and testosterone concentrations than white mothers. Maternal estriol concentrations were lower in primigravidae compared with multigravidae, while nulliparous women (those who had not previously had a live- or stillborn birth) had higher maternal DHEAS concentrations. Maternal estriol levels were higher in pregnancies with a female fetus.

DHEA, DHEAS, estrone and testosterone were lowest in the lightest babies compared with all other birth weight categories (Table 3). This pattern of categorical results was generally similar for birth length. Cord DHEAS was significantly lower in babies born before 38 weeks compared with those born later. Mean testosterone was significantly higher, and mean estriol significantly lower in pregnancies with a male fetus.

Babies delivered vaginally had higher cord levels of androstenedione (357 *versus* 240 ng/dl; $p < 0.001$), estriol (232 *versus* 113 ng/ml; $p < 0.001$) and estrone (34,631 *versus* 17,745 pg/ml; $p = 0.002$) compared with babies delivered by c-section. Length of labor positively correlated with cord DHEA ($r = 0.25$), DHEAS ($r = 0.32$) and estradiol concentrations ($r = 0.23$), and women whose blood was drawn after labor started ($n = 59$) had significantly higher maternal DHEA (603 *versus* 269 ng/dl; $p < 0.0001$), DHEAS (111 *versus* 59.0 ug/dl; $p = 0.0005$) and estrone (7789 *versus* 4675 pg/ml; $p = 0.004$) concentrations compared with women whose blood was drawn before labor started, although the length of labor (before blood draw) did not correlate with any of the maternal hormones (data not shown). Estrone concentrations were higher (28,329 *versus* 16,875 pg/ml; $p = 0.03$) in cord but not maternal serum of pregnancies in which oxytocin was administered at some time prior to delivery.

Adjustment for whether blood was sampled before or after labor started resulted in the attenuation of the association of maternal estrone with maternal age, gestational age and birth weight (data not shown). Table 4 presents the results of the other multiple linear regression analyses that were performed to assess the independence of the associations of pregnancy factors with the maternal hormones. Although most associations remained with mutual adjustment for factors associated with each hormone in univariate analyses, several associations were attenuated. The positive association between birth weight and maternal estradiol was attenuated with adjustment for gestational and maternal age, and the association between maternal pre-pregnancy weight and maternal testosterone was attenuated with adjustment for maternal age, race and maternal height.

Table 4. Results of linear regression models predicting maternal serum hormone concentrations

Dependent variables	Independent variables	Crude β	p	Adjusted[a] β	p
Androstenedione	Maternal age (years)	0.969	0.003	0.971	0.007
	Black *versus* white race	1.51	0.002	1.44	0.002
Testosterone	Maternal age (years)	0.963	0.0006	0.966	0.0006
	Pre-pregnancy wt (kg)	1.01	0.08	1.00	0.13
	Maternal height (cm)	1.02	0.12	1.00	0.74
	Black *versus* white race	1.84	< 0.0001	1.70	< 0.0001
DHEAS	Maternal age (years)	0.959	0.002	0.965	0.02
	Parous *versus* nulliparous	0.857	0.02	0.927	0.30
Estradiol	Maternal age (years)	1.01	0.56	1.00	0.74
	Gestational age (weeks)	1.03	0.22	1.01	0.67
	Birth weight (100 g)	1.02	0.07	1.02	0.15
Estriol	Maternal age (years)	1.02	0.04	1.01	0.48
	Multi- *versus* primigravid	1.06	0.06	1.02	0.60
	Female versus male	1.22	0.07	1.28	0.02
	Gestational age (weeks)	1.08	0.0003	1.06	0.009
	Birth weight (100 g)	1.03	0.001	1.02	0.03

[a] Adjusted models included factors associated with the hormones in correlations and categorical analyses and which remained associated with the hormones after adjusting for non-etiologic factors (timing of blood collection, delivery method, medication use, labor duration). β estimates are mutally adjusted for all variables listed for a particular hormone, and the anti-logs of the β estimates are presented.

Table 5. Results of linear regression models predicting umbilical cord serum hormone concentrations

Dependent variables	Independent variables	Crude β	p	Adjusted[a] β	p
Testosterone	Female *versus* male	0.681	0.01	0.699	0.02
	Birth weight (100 g)	1.02	0.16	1.01	0.30
DHEAS	Maternal age (years)	1.02	0.02	1.02	0.07
	Gestational age (weeks)	1.09	0.002	1.05	0.12
	Birth weight (100 g)	1.04	0.0005	1.03	0.01
Estriol	Female *versus* male	1.42	0.009	1.42	0.009

* Adjusted models included factors associated with the hormones in correlations and categorical analyses, and which remained associated with the hormones after adjusting for non-etiologic factors (timing of blood collection, delivery method, medication use, labor duration). β estimates are mutally adjusted for all variables listed for a particular hormone, and the anti-logs of the β estimates are presented.

The inverse association between parity and maternal DHEAS did not remain after adjustment for maternal age, and positive associations of maternal age and parity with maternal estriol did not remain with adjustment for gestational age, birth weight and fetal sex.

Cord androstenedione, DHEA, estradiol and estrone did not remain associated with any of the factors of interest once adjusted for non-etiologic factors such as timing of blood collection (whether labor started before or after), duration of labor, delivery method and mother's use of oxytocin. Birth weight remained positively associated with cord DHEAS with adjustment, whereas the associations of gestational age and maternal age with cord DHEAS became slightly attenuated (Table 5). Cord testosterone levels remained significantly higher in pregnancies with a male fetus with adjustment for birth weight, but the association of testosterone with birth weight was attenuated. Birth weight was not associated with any of the cord estrogens or androgens besides DHEAS, in the models that included gestational age.

Discussion

Of the associations of pregnancy factors and offspring's subsequent risk of breast and other cancers, the strongest and most consistent in epidemiologic studies appears to be the positive relationship with birth weight [14]. Though widely speculated to be mediated through greater fetal estrogen exposure, few studies have evaluated the consistency of the data with this hypothesis. There is some support for an increase in maternal estrogen levels, in particular, estriol, with increasing birth weight. Kaijser *et al.* [6] recently showed a positive association between birth weight and an estimated measure of cumulative maternal estriol, with levels twice as high at 37 weeks in mothers who delivered babies >4500 g than in those with babies <2500 g. The association was independent of maternal age, smoking during pregnancy, placental weight and ponderal index. Another study found a positive association between birth weight and maternal estriol in US Caucasian women but not in Chinese women [15], and birth weight was positively correlated with total estrogens in a third study [7]. Our maternal data are consistent with these previous findings with estriol levels doubled in mothers with large compared with small babies. In contrast, in our data we did not observe any associations between birth weight and estrogen concentrations in cord serum. Another study [16] also showed no overall association between birth weight and cord venous estriol, and found an inverse correlation in males ($r=-0.32$) when stratified by fetal sex. Birth weight and cord venous estrone and estradiol concentrations in another study also were unassociated [8]. Taken together, these data suggest that maternal estrogen levels may not always be an appropriate surrogate for fetal estrogen exposure.

The fetal testis synthesizes and secretes testosterone and other hormones early in pregnancy, hormone production peaks mid-gestation and declines thereafter. In contrast, the fetal ovary is relatively inactive throughout pregnancy. Cord androgen concentrations generally have been shown to be higher in pregnancies with male fetuses than those involving females [16–19], whereas little difference in estrogens by fetal sex has been observed [18–24]. Our data for cord testosterone concentrations are consistent with this, although it is unclear why estriol levels were elevated in pregnancies with female babies.

Other maternal, perinatal and pregnancy factors have not been well studied regarding their relationships with maternal and fetal hormone concentrations. Henderson *et al.* [3] observed significantly higher maternal testosterone levels in the early pregnancies of black women compared with white women, and hypothesized that this difference might underlie the higher testicular cancer incidence rate in black men. Our results for maternal

testosterone and androstenedione concentrations late in pregnancy agree with their data [3], however, as was the case for birth weight, racial differences in hormones were not demonstrated in cord blood. We found that maternal androgens and cord DHEAS decreased with maternal age, whereas it was unassociated with maternal or cord estrogens after adjustment for other factors. While we are unaware of other data on maternal age and androgens, maternal age was positively associated with maternal estriol concentrations in one study [6], but the association did not remain with adjustment for birth weight and smoking. Positive associations between maternal age and cord estrone and estradiol were demonstrated in another study [8], although these were unadjusted for other factors. The results of a third study on maternal age and estrogens were ambiguous [9]. In our data, maternal body size was unassociated with estrogens, and a positive correlation between height and maternal testosterone was completely attenuated with adjustment for maternal age and race. Bernstein *et al.* [10] found a positive association between pre-pregnancy weight and free estradiol at the end of the first trimester, while another study conducted later in pregnancy found inverse correlations between pre-pregnancy weight and maternal estradiol, and between maternal height and maternal estradiol and estriol [15]. We found no evidence of a smoking-hormone relationship, in contrast with previous studies observing lower estrogens in mothers who smoked during pregnancy [6, 7, 25]. Our variable for smoking, based on whether the mother smoked at any point during pregnancy, likely included a high proportion of women who smoked only minimally, and may have biased our results toward no association. Higher estriol concentrations in mothers in our study experiencing their first pregnancy compared with mothers experiencing subsequent pregnancies disappeared with adjustment for other factors related to maternal estriol including, maternal age, gestational age, birth weight and fetal sex. In another study comparing mothers early in their first and second pregnancies, total estradiol remained elevated in the first pregnancy, after accounting for maternal age and pre-pregnancy weight [10].

The data on which this epidemiological analysis was based were collected in a clinical study and the response rate in the controls was low. The paucity of literature on the associations of putative perinatal risk factors and umbilical cord blood hormone concentrations, however, led us to believe this was a reasonable group to use to set priorities for more robust work in testing hypotheses. Random measurement error in the hormones may have resulted in the lack of association between certain factors and maternal and cord hormone concentrations in our data. The combined inter- and intraassay laboratory errors, calculated using blinded replicates, were over 10% in several cases. Differences in the size of the coefficients of variation between the maternal and cord samples were not large for estriol (13.7 *versus* 10.9%, respectively) and estradiol (6.8 *versus* 9.2%, respectively), however, and would not explain the lack of association of pregnancy factors with cord hormones since associations were found with maternal concentrations. In contrast, the large number of comparisons may have resulted in some statistically significant results entirely by chance. While our maternal data were collected close in time to delivery, we attempted to reduce bias from extraneous factors associated with labor and delivery by adjusting for them in the analyses.

In conclusion, this study demonstrates associations between pregnancy factors and maternal and cord hormone concentrations that have been reported previously, including positive associations between birth weight and maternal estriol, and elevated androgens in black mothers. The data, however, do not provide evidence of these associations with hormones measured in the fetal circulation. Our data on hormone concentrations in the fetal circulation are not consistent with the hypothesis that greater estrogen concentrations in high birth weight babies mediate the positive association with breast cancer risk observed in epidemiologic studies, or with the hypothesis that elevated testosterone levels in the *in utero* environment of black males explains their higher subsequent prostate cancer risk. Further studies are warranted to identify variation with respect to pregnancy characteristics in hormones measured in cord blood, with the prospect of relating these potential markers to subsequent cancer risk in offspring.

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